Insulin Secretion in the Obese (ob/ob) Mouse

The Effect of Oxytetracycline on Insulin Release

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SUMMARY

The effect of oxytetracycline (OTC) pretreatment on the response of the ob/ob mouse to insulin secretagogues in vivo and in vitro was investigated. With glucose loading in vivo, the peak glucose was twofold greater and the insulin levels threefold greater in obese than in lean mice. After OTC treatment, there was no significant difference in insulin levels between lean and obese mice although the peak glucose level was still 1.5 times as high. Glucagon increased plasma glucose 2.5-fold and plasma insulin 20-fold in the obese as compared with lean mice. After OTC treatment, the glycemic response of the obese was indistinguishable from that of the lean control. The insulin levels, while higher than those of lean mice, were only 25% of those found in the untreated obese. Aminophylline produced an 8- and a 20-fold increase in peak glucose and insulin levels, respectively, as compared with lean mice. In the OTC-treated obese mice, the injection of aminophylline produced a slower rise in plasma glucose than in the obese controls, but the levels were not significantly different from those of the untreated obese mice at 90 min. On the other hand, the insulin levels attained a plateau at a value which was one-fifth that found in the control obese group. In vitro, isolated islets from obese mice showed an exaggerated response to the secretagogues. Pretreatment with OTC attenuated this response. The fraction of insulin released at 10 mM glucose was less than one-fourth that in the obese controls. With glucagon added, the response was only one-eighth, and with aminophylline, one-half as great in the OTC-treated than in the obese control. The effects of OTC cannot be attributed to the effects of the drug on food consumption, since obese mice food restricted to the intake of the OTC-treated obese mice showed either no improvement or much smaller changes. **DIABETES *32*:932–937, October 1983.**

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Received for publication 1 November 1982 and in revised form 28 March 1983.

The ob/ob mouse has been used extensively as a tool to study obesity and diabetes syndromes. This animal model is characterized by hyperphagia, obesity, nonketotic hyperglycemia, hyperinsulinemia, islet cell hyperplasia, and resistance to insulin.[1] Insulin secretion in response to a variety of stimuli is exaggerated in the ob/ob mouse, whether the stimuli are applied in vivo or in vitro.[2–6] Qualitatively, the response to glucose is normal and the two phases of insulin secretion well delineated (cf., Figure 4 of ref. 3). Quantitatively, the response is out of proportion to the stimulus applied.[3,5]

We have used the antibiotic oxytetracycline (OTC) as a tool to study the metabolic defect in the ob/ob mouse.[7–9] This compound potentiates the action of insulin[10–12] and insulinotropic agents [13,14] in vivo. OTC treatment corrects many of the metabolic abnormalities of the obese mouse. It decreases body weight, lipid content of tissues, and plasma glucose and insulin levels.[7] Insulin sensitivity is restored to the muscle[8] and the binding of insulin by liver membranes is enhanced.[7] The finding that the islets of the obese mouse are regranulated following OTC treatment[9] led us to investigate whether the antibiotic exerts some of its effects on islet cell function as well as on peripheral tissues.

MATERIALS AND METHODS

Animals. Male C57BL/6J-ob/ob mice and their lean controls (+/?) were obtained from the Jackson Laboratory (Bar Harbor, Maine). The mice were used in the various experiments at 9–12 wk of age. Unless otherwise specified, they were maintained on Purina Chow and water ad libitum. The animals were divided into the following groups: (1) lean control, (2) lean OTC-treated, (3) obese control, (4) obese OTC-treated, and (5) food-restricted obese mice.

Treatments. Groups of lean and obese mice were treated for 7 days with oxytetracycline (Terramycin, Pfizer) via intra-

muscular injections as described previously.[7] The last injection of OTC or saline was administered at 1600 h the day before each experiment. The dosage of OTC (100 mg/kg) was calculated on the basis of the body weight of the untreated lean mice. The controls were injected with saline. In the experiment described in Figure 1, half the animals in the obese control group were injected with saline and the other half with lidocaine, at the concentration present in the commercial Terramycin preparation, to control for possible effects of the anesthetic. There were no significant differences between the two sets of data; therefore, the results were pooled. In subsequent experiments, control mice were injected with saline. The food restriction schedule consisted of feeding groups of obese mice an amount of food equivalent to the average amount consumed by the OTC-treated obese mouse. This has previously been found to be equal to approximately 4.5 g/day.[7] The weighed food was placed in the appropriate cages at the same time as the OTC injections were given (1600 h).

In vivo response to secretagogues. Twelve hours before each test, at 2100 h, food was removed from the cages. At 0900 h the following day, blood samples were taken from the tail vein into heparinized capillary tubes (0 time sample), test compounds were injected intraperitoneally at the concentrations indicated in the legends to the figures and blood samples were withdrawn at 5, 15, 30, 60, and 90 min following the injection of the test compound. The experiments were performed without anesthesia.

Glucose determinations. After separation of the plasma by centrifugation a 5-μl portion of plasma was diluted with 10 μl H_2O and used for the determination of glucose by a glucose-oxidase method using the Beckman Glucose Analyzer. Incubation media used for the isolated pancreatic islets were checked by the same method.

Insulin determinations. These were done on plasma by a modification[15] of the double antibody radioimmunoassay of Hales and Randle,[16] using rat insulin as a standard. The method was equally valid for heparin and EDTA-plasma or serum. The same method was validated for use in the determination of insulin on islet cell incubation media and on supernatant from islet extracts.[15] Glucagon (9 μU insulin/mg, from Lilly Research Lab) and aminophylline at concentrations up to 100 times those used in islet incubation media were tested for interference in the insulin RIA; they were found to have no effect.

Preparation of islets. Fed mice were killed by decapitation between 0900 and 1000 h. Islets were prepared by a method based on the collagenase method of Lacy and Kostianovsky.[17] Essentially, a mid-line incision was made and the pancreas rapidly covered with ice-cold Krebs Ringer bicarbonate buffer (KRB) containing 3 mM glucose. It was then removed, freed from the spleen, fat, and connective tissue, and minced into small pieces with scissors. Four to five tissues were processed as a batch. The pancreas pieces were washed twice with fresh cold buffer and twice with buffer at 37°C (warm KRB). The washed pancreas pieces were then placed into the digestion buffer. For each pancreas, 10 mg of collagenase and 5 mg of hyaluronidase were added to 0.5–0.8 ml of warm KRB. The slurry was shaken vigorously by hand at 37°C for 5–8 min. The reaction was stopped by the addition of 100 ml cold KRB containing 1 mg/ml bovine serum albumin. Islets were isolated from the digest and placed in a common pool before selection for static tube incubation. Each tube received five well-preserved islets. The tubes were selected at random for different treatments. The composition of the KRB buffers was as follows. The cold KRB contained (in mmol/L) Ca^{2+}, 2.7; Mg^{2+}, 1.26; Na^+, 139; K^+, 6.22; Cl^-, 134.4; SO_4^{2-}, 1.26; $H_2PO_4^-$, 1.26; HCO_3^-, 63.5. The warm KRB had the same composition except that the concentration of HCO_3^- was 15 mM. The pH of both buffers was adjusted to 7.35 by saturating with CO_2.

Measurement of insulin secretion. The tubes containing the islets were preincubated for 45 min in a medium consisting of the warm KRB buffer containing 1 mg/ml bovine serum albumin and 3 mM glucose. After replacement of the preincubation medium with fresh medium, the islets were incubated at the desired concentration of the secretagogues for a period of 60 min. Throughout the preincubation and incubation, the islets were maintained in an atmosphere of 95% O_2/5% CO_2. At the end of the incubation period, the medium was removed and frozen for subsequent determination of insulin. After washing three times with fresh buffer, the islets were extracted with acid ethanol.[18] After appropriate dilution, these extracts were used for the determination of insulin.

Expression of results. In dealing with samples from lean and obese mice, one is confronted by difficulties in the selection of a method for the expression of results. The islet populations in the two groups of animals differ in size, shape,

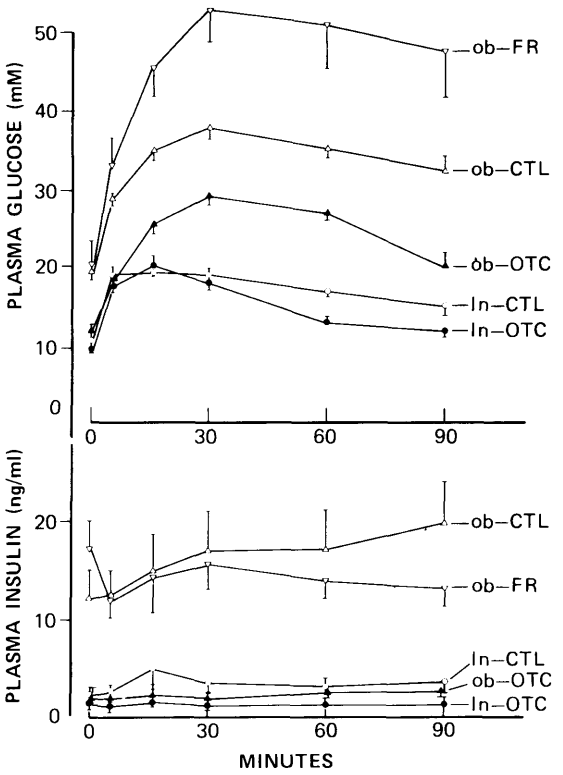

FIGURE 1. Plasma glucose and IRI following a glucose load (1 g/kg). The mice were prepared and injected as described in METHODS. Top panel, plasma glucose; bottom panel, plasma IRI. Results are given as means ± SEM, N = 8 in each group. Lean control, —○—; lean OTC-treated, —●—; obese control, —△—; obese OTC-treated, —▲—; obese food-restricted control, —▽—.

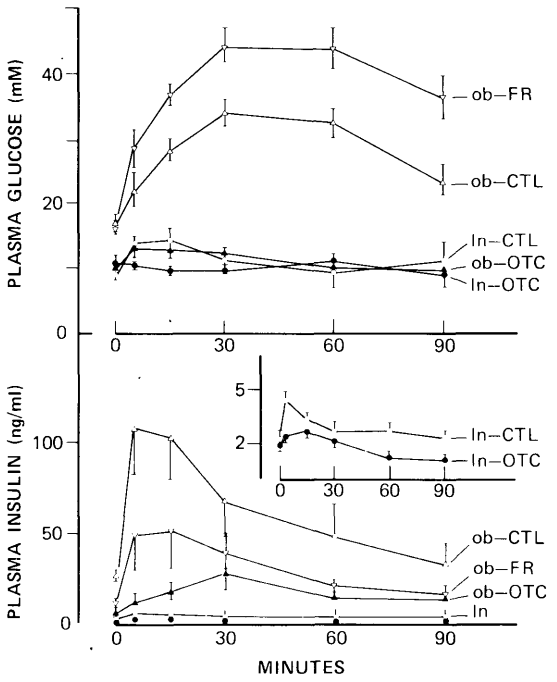

FIGURE 2. Plasma glucose and IRI following a glucagon injection. Each mouse received 25 μg glucagon. Other details were as given in the legend in Figure 1.

and degree of granulation. In addition, whereas in the lean mice the islet cell population is fairly homogeneous, in the obese, within one pancreas, islets of different sizes and/or different degrees of granulation are found. Since insulin secretion is highly correlated with insulin content of the islets,[19] it was decided to express the results as insulin secreted per unit time as a fraction of the total insulin content of the islets, a method which has been used by others.[20-23]

Statistical analyses. Results are expressed as mean ± SEM. For the in vivo experiments, N = number of animals, while for the in vitro experiments, N = number of batches of islets (5 islets/batch). The day-to-day variation in the total amount of insulin secreted by different batches of islets studied under the same conditions was assessed by calculation of the coefficient of variation, which was less than 5%. Student's unpaired *t* test was employed as the test of significance.

Materials. Collagenase and hyaluronidase were obtained from Worthington Biochemical Co. Ltd (Freehold, New Jersey); bovine serum albumin (fraction V RIA grade) and aminophylline were from Sigma Chemical Co. (St. Louis, Missouri); oxytetracycline (Terramycin) was from Pfizer (Montreal, Canada). The rat insulin standard was purchased from Novo Industries AS (Copenhagen, Denmark). The glucagon used for the in vivo tolerance tests was obtained from Connaught Laboratories (Toronto, Canada). The glucagon for the in vitro experiments (Lot No.: 258-25J-120) was a gift from Lilly Research Labs (Indianapolis, Indiana).

RESULTS

IN VIVO EXPERIMENTS

Effect of glucose. Figure 1 represents the response of lean and obese mice to the intraperitoneal injection of glucose and serves as a landmark for evaluating the action of other secretagogues. As observed previously under somewhat different experimental conditions,[7,9] OTC treatment partially restored the ability of the obese mouse to handle a glucose load. At all times studied, the plasma glucose levels were significantly lower (P at least <0.05) in the OTC-treated than in the control obese mice. The plasma glucose levels at 0 time and at 5 min were similar in the OTC-treated obese and in both groups of lean mice. They continued to rise until the 30-min point in the OTC-treated obese, whereas the peak was reached at 15 min in the lean. The OTC treatment decreased the levels of insulin in the obese mouse to the levels observed in the lean controls. The insulin levels in the OTC-treated lean mouse were also decreased as compared with the control. Furthermore, the insulin curves in both lean and obese OTC-treated mice did not show a peak of insulin secretion such as was found in the lean controls. The results in Figure 1 indicate clearly that OTC did not produce its effects by decreasing the food consumption of the obese mouse. In the food-restricted mice, a glucose load produced marked and sustained hyperglycemia and insulin secretion was similar to that of the controls.

Effect of glucagon. In the obese mouse, the effect of previous OTC treatment on the response to an acute injection of glucagon was striking. In the obese control and obese food-restricted groups, the glucagon injection elicited an exaggerated elevation of the blood glucose levels but in the OTC-treated obese mouse the effect of glucagon was not significantly different from that found for the lean mouse. In the OTC-treated lean mice, a glucose peak was not ob-

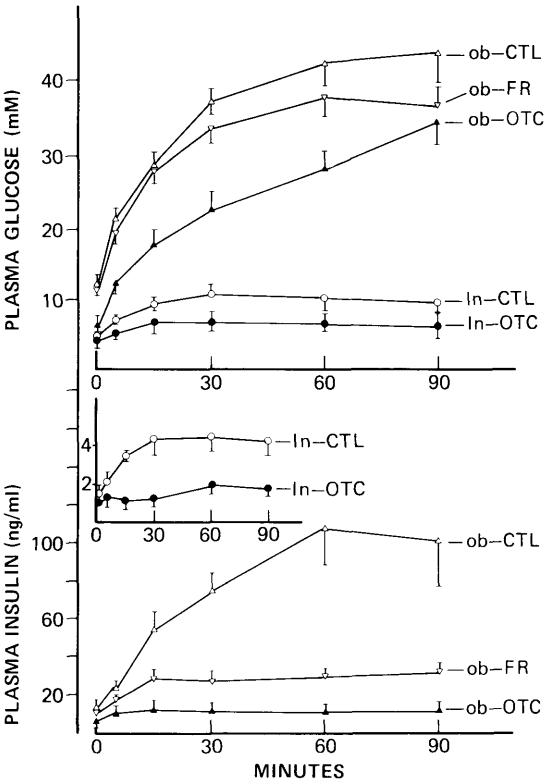

FIGURE 3. Plasma glucose and IRI following an injection of aminophylline. Each mouse received 3 mg aminophylline. Other details were as given in the legends to Figures 1 and 2.

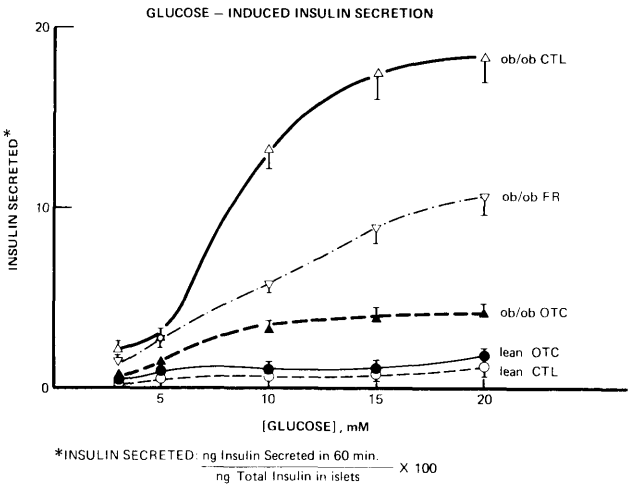

FIGURE 4. The release of insulin by isolated islets of Langerhans. The islets were preincubated in 3 mM glucose as described in the text. They were incubated for 60 min at the glucose concentrations indicated on the abscissa. Insulin measurements and calculations were done as described in METHODS. Results are given as the means ± SEM of 15–20 batches of islets (5 islets/batch). The average insulin content of pancreatic islets in ng/islet was: lean controls, 34 ± 2; lean OTC-treated, 32 ± 1.5; obese controls, 16 ± 2; obese food-restricted, 43 ± 3; and obese OTC-treated, 86 ± 7.

served, and at 5 min, the serum glucose concentration was significantly lower than in the lean controls (Figure 2). The effects on insulin levels were varied (Figure 2). Both the lean and obese control groups showed a peak value around 5 min and a return toward 0 time levels within 90 min after the injection of glucagon. The return to the 0 time level was, however, much more rapid in the lean mice. It should be noted also that, although the changes were qualitatively similar in the lean and obese mouse, the levels were at least 10 times greater in the latter.

There were two distinct effects of OTC treatment on insulin secretion. First, the peak of secretion seen at 5 min in the controls was virtually absent in the OTC-treated animals, both lean and obese. Second, in the early part of the experiment, both the lean- and the obese-treated mice had insulin levels which were significantly lower than those of their respective controls. Food restriction, although (or perhaps because) it diminished insulin secretion, led to increased blood glucose levels throughout the duration of the test.

Effect of aminophylline. The responses to aminophylline are shown in Figure 3. In both control and treated obese mice, an acute aminophylline injection produced a rapid and sustained elevation of the blood glucose levels. The blood glucose levels were, however, significantly lower in the OTC-treated than in the control obese mice at all times except for the 90-min sample. In the obese control mouse, this sustained hyperglycemia occurred in spite of sustained hyperinsulinemia, whereas in the OTC-treated obese mouse, the insulin levels, while still elevated compared with the values seen in the lean mouse, were at least 5 times less than those seen in the obese control mouse. In the lean mouse, the levels of glucose and insulin were both proportionately higher in the controls than in the OTC-treated mice. In both the latter groups, the increases were relatively modest in comparison to those seen in the obese mice. Again food restriction of the obese mice diminished the secretion of insulin but not the production of glucose.

The obese mouse is extremely sensitive to methylxanthines.[24] In our hands, 50% of the obese mice (all groups) died within 48 h following the test, although they did not show signs of distress during the performance of the test itself.

IN VITRO EXPERIMENTS

Effect of glucose on insulin secretion. The results of these experiments are given in Figure 4. Compared to the tissue from the lean mice, the islets from the control obese mice secreted exaggerated amounts of insulin at all the glucose concentrations studied. Treatment of the obese mouse with OTC led to a significant decrease of insulin secretion. The insulin secretory activity of the OTC-treated obese mouse islets was still significantly greater than that of the lean mouse. Restricting the food intake of the obese mouse to the levels consumed by the OTC-treated obese mouse did not decrease insulin secretion as much as did the OTC treatment.

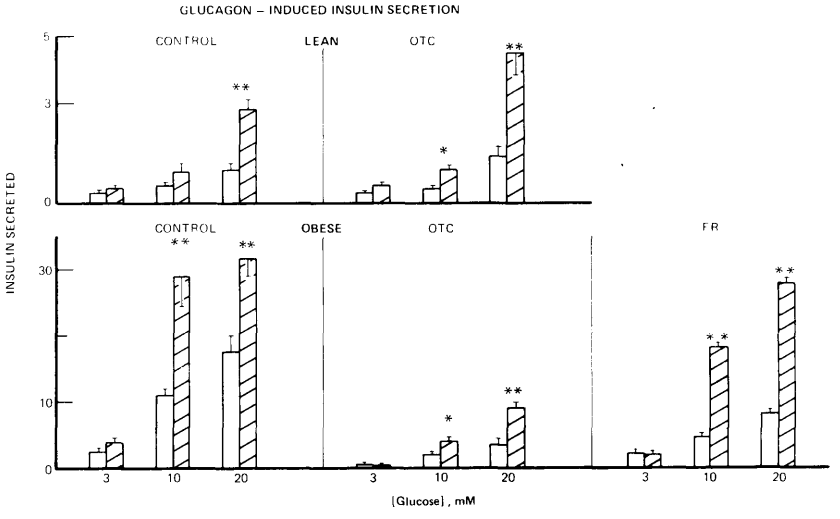

FIGURE 5. The effect of glucagon on insulin secretion by isolated islets. The release of insulin was measured in presence (hatched bars) and in absence (open bars) of glucagon (5 μg/ml). The conditions were as described in METHODS. Results are given as the means ± SEM of 10–15 batches of islets.* $P < 0.05$, $P < 0.005$, between glucose alone and glucose + glucagon.**

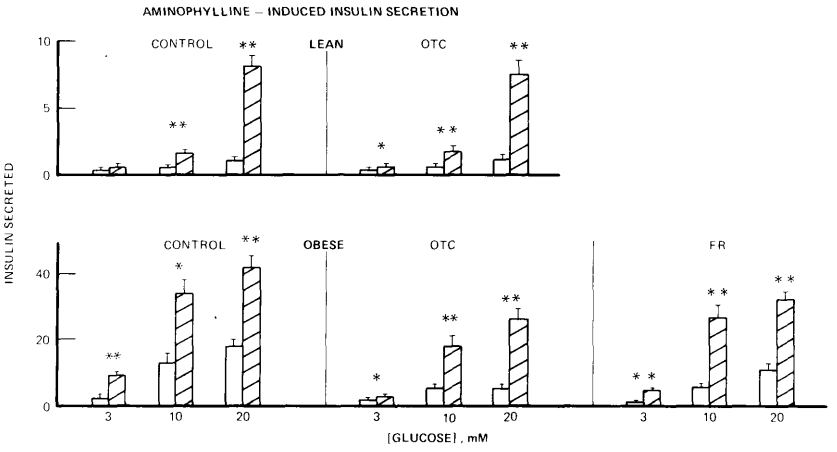

FIGURE 6. The effect of aminophylline on insulin release by isolated islets. The release of insulin was measured in the presence (hatched bars) and in the absence (open bars) of aminophylline (2.5 mM). The conditions were as described in METHODS. Results are given as the means ± SEM of 10–15 batches of islets. *P < 0.05, **P < 0.005, between glucose alone and glucose + aminophylline.

Effect of glucagon. The addition of glucagon to the incubation medium at various glucose concentrations produced a modest stimulatory effect in the islets from the control lean mice. At glucose concentrations of 10 mM and below, glucose alone or glucose + glucagon had little effect on the islets from lean mice previously treated with OTC. The stimulation produced by both secretagogues was significantly higher than that found in the control mouse when the glucose concentration of the medium was 20 mM (Figure 5). In the control obese mouse, the already exaggerated insulin secretion due to glucose was boosted even further by the addition of glucagon. In the OTC-treated obese mouse, there was a modest increase caused by glucagon. Food restriction of the obese mouse did not lead to any significant improvement in the pattern of insulin release when glucagon was combined with 20 mM glucose.

Effect of aminophylline. There were no significant differences in the response to aminophylline of the islets of lean control or lean OTC-treated mice (Figure 6). In the islets of the obese (control, food-restricted, and OTC-treated) aminophylline stimulated the secretion of insulin maximally at 10 mM glucose. The fraction of insulin secreted by the islets of the OTC-treated mice was, however, significantly smaller than that in the islets of the control obese mice.

DISCUSSION

The primary purpose of these studies was to determine whether the effects of OTC on the function of pancreatic islets[9] resulted from the improvement of insulin sensitivity of the peripheral tissues[7,8,10–14] or whether they were due to more specific effects on the beta-cell.

The secretagogues used (glucose, glucagon, and aminophylline) were chosen because of their diverse modes of action in promoting insulin secretion. Although, ultimately, they probably all influence Ca^{2+} movements, they do so via different mechanisms.[25–32] Glucose or, more likely, one of its metabolites is thought to mediate insulin secretion via effects on the uptake and redistribution of Ca^{2+} in the beta-cell. Glucagon is believed to act by increasing cAMP levels. Aminophylline, like glucagon, could act via increased cAMP levels. The action of the methylxanthines is very complex and, in addition to their inhibitory effects on phosphodiesterase, some of their effects may be due to interactions with the adenosine receptor.[33] Aminophylline is particularly interesting in view of previous reports of the extreme sensitivity of the ob/ob mouse to caffeine, another methylxanthine.[24]

OTC does increase the sensitivity to exogenous insulin in the absence of functional beta-cells,[10–12] presumably via effects on the peripheral tissues. However, both previous work and the results presented here support the conclusion that OTC also has a more direct effect on the beta-cell. The time course of OTC effect is such that a fall in circulating insulin levels accompanied by an increase in insulin receptor activity precedes the return of plasma glucose levels to normal values.[7] If the increased sensitivity of the periphery were the primary factor in the return to normal of the glucose and insulin levels, a fall in the plasma glucose levels would be expected to occur before, or at least at the same time as, the decrease in plasma insulin levels.

Tetracycline added in vitro has been shown to inhibit glucose-stimulated insulin release in isolated rat islets in vitro. The inhibitory effect of the drug was apparent at 2–20 μM but not at 200 μM,[34] probably due to a combination of the chelating and ionophoretic properties of the drug.[35] Chlortetracycline has also been shown to interact with specific calcium pools in intact islets and in collagenase dispersed islets of noninbred obese mice.[35] The effects which we observed might therefore represent an inhibition of insulin secretion due to the chelation of essential calcium ions. This is an unlikely explanation because islets isolated from both lean and obese mice treated with OTC responded appropriately to the stimuli applied, ruling out a direct inhibitory effect of OTC on insulin secretion. There was little difference in the response of the two groups of lean mice (control and OTC-treated) to the various secretagogues. This indicated, by analogy, that the decrease in insulin secretion produced by OTC in the obese mouse was not a simple inhibition due to the removal of some essential component (e.g., calcium) from the islets. Another argument in favor of islet-specific effects of OTC is that food restriction which also diminished the demand for and the secretion of insulin in the obese mouse did not lead to regranulation of the beta-cell.[9] In addition, while food restriction significantly decreased the in vitro insulin secretory response evoked by glucose, there was no difference between the control and the FR group when more powerful secretagogues such as glucagon and aminophylline were used in conjunction with glucose.

These results show that in the obese mouse, treatment

with OTC diminished the insulin secretory activity of the beta-cell independently of plasma glucose levels, and in spite of an accumulation of insulin in the beta-cell. A hypothesis now being tested to explain the action of the antibiotic is that it may increase islet insulin receptor activity,[36,37] as it increases receptor activity in the liver,[7] and thus, it may promote feedback inhibition of insulin secretion by insulin itself.[38,39] This process is reportedly lost or diminished in the obese mouse.[40] This hypothesis offers the advantage of reconciling the peripheral effects of OTC with those found on islets.

ACKNOWLEDGMENTS

The authors are indebted to Dr. John Logothetopoulos for teaching them the art of islet preparation.

This work was supported by grants from the Canadian Diabetic Association and from the Foundation of the Children's Hospital of Eastern Ontario. MDS is the recipient of a Studentship from the Medical Research Council of Canada.

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